

DEFINING A NEW FORENSIC DISCIPLINE: MICROBIAL FORENSICS

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INTRODUCTION

A number of bacteria, viruses, and fungi pose serious health concerns to humans, threaten the U.S. agricultural economy and food supplies, and/or affect the environment. The potential for use of any of these pathogenic agents as a biological weapon has been demonstrated (recently with *Bacillus anthracis* and many times in history), and further attacks cannot be ruled out. Microorganisms make particularly good weapons because pathogenic agents can be grown from a single organism or cell and often inexpensively. Moreover, it may be impossible to identify when very small amounts of such materials have been removed surreptitiously from a facility that is authorized to possess pathogens. Even the smallest amount can become a major threat.

Obviously, better controls are needed to protect legitimate users and to deter or prevent criminal dissemination of dangerous microorganisms or their toxic by-products. Better information and/or access to information is required on those individuals who have access to these pathogens so threats can be deterred or effectively traced back to possible sources. Such information can direct law enforcement officials to those with expertise on specific threat agents for assistance. Should there be an incident in which a pathogen is deliberately released in the U.S., law enforcement needs the means to rapidly and efficiently identify all U.S. facilities (if any) that legitimately hold that pathogen, those facilities that hold the pathogen with that specific genetic profile, and those individuals who have had access to the specific microorganism. To achieve this goal, a national database(s) of pathogens, pathogen profiles and individuals authorized to have access to these pathogens must be established.

Critical to the success of law enforcement investigations and criminal prosecutions is the development and validation of methods to type the various threat agents in ways that can be used to attribute criminal acts. Forensic requirements and standards for microbial analyses go beyond those typical for licensing of clinical microbiology tests for human, animal or plant health purposes. Orchestration of the tasks required to establish a national microbial forensics program falls most appropriately under the purview of the Federal Bureau of Investigation. The FBI has the existing infrastructure, investigative responsibility, forensic expertise and demonstrated leadership in forensic genetic identification technologies. Lastly, it would be desirable to extend capabilities to encompass microorganisms studied worldwide (a more daunting task).

NEEDS

For Law Enforcement agencies to investigate, attribute, deter and prevent biological threats and for the Justice Department to prosecute criminals, the field of Microbial Forensics must be developed. In order to establish the field and needs of Law Enforcement, the following short- and long-term goals must be met:

1. The FBI will support and host a Scientific Working Group on Microbial Genetics and Forensics (SWGGMGF) to facilitate addressing the goals stated below. The SWGGMGF processes will be modeled after the successful Scientific Working Group on DNA Analysis Methods for human forensic DNA analyses. One key to the success of this new group is to bring together people who do not normally get together to address these issues. The combined multi-disciplinary expertise of the SWGGMGF participants will be used to develop a peer consensus on various microbial forensic topics. Members will be recognized experts predominately from government, but with selected participants from academia. Private industry will be called upon to advise as needs demand. This has been a proven mechanism for developing a high quality system and should be supported.

2. Federal agencies must develop, maintain and regularly update prioritized lists of biological agents that have the potential to cause severe harm to humans, animals, plants or the environment and materiel resources. The list(s) should be categorized based on pathogenic properties and specific intelligence on possible threats. The SWGMGF will establish threat microorganism criteria and prioritize the threat microorganisms. Therefore, efforts can be focused to generate data and develop methods of analysis that are most urgently needed. These prioritized microorganisms, determined by interagency input, should be reviewed annually and more frequently if new intelligence is obtained.

3. The requirements and framework for a DNA database that will contain all available relevant biologic and genetic information on threat agents should be established by SWGMGF, so that, where possible, definitive organism, strain, and/or sub-strain identification may be carried out. Such data will support investigations attempting source attribution and, hopefully, distinguish genetically engineered strains from those that occur naturally in the environment. The database must have the capacity to store full genome sequence data from many organisms and allow these data to be searched in a rapid manner to compare one whole genome sequence with another (or a portion of one genome with another). Once developed, specific assays, that do not require full genome analysis, can be compared. The database will be used as a reference during investigations, as well as a baseline for development of assay and technology transfer.

Obviously, policy decisions must be made on the location and security requirements for the database. Data will need to be stored and analyzed in both classified and unclassified settings, and the analytical processes will require high computational power (e.g., an ASCI-class supercomputer). A location for the database could be one of the National Laboratories.

4. Considerable new research must be carried out to provide the information necessary to profile pathogens. Briefly, the genetic sequences of sufficient strains of the threat agent (e.g., *Bacillus anthracis*) must be determined so that one can identify the microorganism conclusively and distinguish it from other life forms (including near relatives such as *Bacillus subtilis*). At the same time, the genetic sequences need to be searched for areas of sufficient variation so that, if possible, one isolate can be distinguished from another. Initial genome sequencing is an easier task for viruses, whose genetic sequences are typically less than 200,000 base pairs in length, compared with bacteria and fungi. It is a more laborious and expensive task for a bacterium with a genome of 3 to 5 million base pairs. The fungi, at 30 to 40 million base pairs per genome represent a much greater level of effort.

When possible, different genomes will need to be sequenced and multiple isolates of each genome will need to be sequenced to provide the information necessary if isolate individualization is to be feasible or provided. Considerable computing power will be needed to handle the information needs, as described above. New and more rapid DNA sequencing techniques are expected that will reduce the effort required. However, the process should begin now with available technologies. The SWGMGF will define criteria for what general species, strains, and isolates should be analyzed.

5. No single federal agency has the necessary complete capabilities to carry out microbial forensics development and validation of assays. The FBI has expertise in human forensic DNA analysis but does not have capability or capacity for work with the multiplicity of microorganisms that must be analyzed. Thus, other agencies and institutions must be called upon to carry out microbial research. These include academia, industry, and a number of federal laboratories, such as the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), the U.S. Department of Agriculture (USDA), the National Laboratories, and others. As an example, the USDA should be responsible for animal and plant threat agents: the animal threat agents foreign to the U.S. must be handled in restricted USDA biocontainment facilities. Also, the National Academy of Sciences will participate in SWGMGF deliberations, and, may undertake independent review of poignant topics.

It is important to realize that the entities that perform the microbial forensics research must do so in close cooperation with law enforcement so that the methods and data are accepted and validated for forensic

uses. Furthermore, these entities must stand ready to assist the FBI in any future enquiries by providing facilities, personnel and analytical capabilities that will form the basis for attribution and prosecution. These are not functions easily contracted to multiple performers on a short term. The SWGMGF will provide an avenue for communication and facilitate relationships.

6. The SWGMGF will develop criteria for a data registration database for users in government, academia, clinical laboratories and private institutions. This database will capture information on facilities, threat agents, personnel access to threat agents, and microorganism identity as defined by existing technologies. The information will be used for investigative purposes and also to identify intellectual resources if an event occurs where specialists are needed. The database must be relational and secure. When available, data from outside the United States should be entered into the database. Thus, SWGMGF should make efforts to foster international relationships. Additionally, to avoid unnecessary duplication of research efforts, current information on threat agent strains that have been or are being sequenced should be gathered along with the details of established typing methods. When designed, the database software and operating system will have to be written and tested. This database will require policy level decisions on access and security.

7. A National Strain Repository must be developed similar to the military's DNA identification program and the U.S. system for protection of patented microorganisms. All U. S. laboratories working with threat agents would be required to provide a sample to be stored in the National Strain Repository along with the genetic details. (This is already done for patented microorganisms and thus models exist). The SWGMGF will recommend procedures and policy options. Policy level decisions must be made on security and access to the National Strain Repository.

8. More and better analytical tests are needed to assess the veracity of a threat at some crime scenes. Currently, there are an insufficient number of validated analytical tools to identify many of the most important threat agents rapidly in the field at the site of an incident. Available tests often lack insufficient genetic information to identify agents down to the strain or sub-strain level. Moreover, some of the tests that are being proffered have not been subjected to validation studies. Therefore, the information derived from analyses in some situations may not be reliable or meaningful. A system will be developed so that analytical tests can be evaluated for reliability, sensitivity, and specificity. The SWGMGF will recommend improvements.

9. Quality Assurance (QA) and Quality Control (QC) measures must be enacted along with standard operating protocols, appropriate controls, and proficiency testing to ensure consistency and validity of the analytical and identification processes (similar to ISO 17025 standards, ISO 9000 standards, and/or FBI Director Standards for DNA Typing). Typing methodologies will be developed and validated for each threat agent using QC and QA criteria to ensure validity of the results. Attenuated or inactivated strains of threat agents are needed for proficiency testing and to serve as assay controls. With defined QA and QC guidelines, the users, the legal community, and the public can have confidence in the results obtained from a crime scene.

10. Training tools on microbiology, safety, methodology, and handling and shipping must be developed for the general public so that all can be better informed. As an example, it is expected that public officials will convey pertinent information regarding the status of a biological effect and what actions the civilian population should take. The training materials could be used to properly guide our public leaders.

11. Genetic analyses are not the only way to identify a microorganism or provide forensic attribution. Such topics as trace material analysis, immunology, culturing, and so forth also need to be addressed. The SWGMGF will address these other scientific issues and needs as they arise.

ADDITIONAL BENEFITS

While the main goal of this microbial forensics program is to support law enforcement, additional benefits can be realized. The genetic database will provide baseline information that will foster technology

development in the field of genetics, improve clinical diagnostics for health care and agriculture, and develop assays that may be used to screen imports that may be contaminated with infectious agents.

CONCLUSIONS

Forensic investigations use physical evidence to attempt to obtain information on the microorganism, the persons involved and places involved, the processes and instrumentation used to develop or disperse the weapon, and/or time of the criminal act. Because of the advances in technology, challenges to national security will continue to arise. The SWGMGF will promote an integrated national (and international) effort, stressing high quality, so bioterrorism challenges can be met rapidly and effectively.

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